Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Glycan processing in gut microbiomes

Sabina Leanti La Rosa[1], Matthew P Ostrowski[2], Arturo Vera-Ponce de León[3], Lauren S McKee[4], Johan Larsbrink[5], Vincent G Eijsink[3], Elisabeth C Lowe[6], Eric C Martens[2] and Phillip B Pope[1,3]

Microbiomes and their enzymes process many of the nutrients accessible in the gastrointestinal tract of bilaterians and play an essential role in host health and nutrition. In this review, we describe recent insights into nutrient processing in microbiomes across three exemplary yet contrasting gastrointestinal ecosystems (humans, ruminants and insects), with focus on bacterial mechanisms for the utilization of common and atypical dietary glycans as well as host-derived mucus glycans. In parallel, we discuss findings from multi-omic studies that have provided new perspectives on understanding glycan-dependent interactions and the complex food-webs of microbial populations in their natural habitat. Using key examples, we emphasize how increasing understanding of glycan processing by gut microbiomes can provide critical insights to assist 'microbiome reprogramming', a growing field that seeks to leverage diet to improve animal growth and host health.

Addresses
[1] Faculty of Biosciences, Norwegian University of Life Sciences, Ås, 1433, Norway

[2] Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, 48109, MI, USA
[3] Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, 1433, Norway
[4] Division of Glycoscience, Department of Chemistry, KTH Royal Institute of Technology, AlbaNova University Centre, Stockholm, 106 91, Sweden
[5] Division of Industrial Biotechnology, Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, 412 96, Sweden
[6] Biosciences Institute, Newcastle University, Newcastle, UK

Corresponding author:
La Rosa, Sabina Leanti (sabina.leantilarosa@nmbu.no)

Current Opinion in Microbiology 2022, **67**:102143

This review comes from a themed issue on **Microbiota**

Edited by **Lindsay Hall** and **Melanie Schirmer**

For complete overview of the section, please refer to the article collection, "Microbiota"

Available online 23rd March 2022

https://doi.org/10.1016/j.mib.2022.102143

Introduction

The gastrointestinal tract of the vast majority of bilaterians is home to densely populated and diverse microbial communities, including bacteria, archaea, fungi, protozoa, as well as a multitude of viruses. Interactions between these trillions of individual prokaryotic and eukaryotic cells and the various dietary components determine important nutritional and immune functions of the host that are tightly linked to health and disease [1,2,3•,4•]. Assembly of gut microbial consortia begins at birth [5] and, over time, their ecophysiology is primarily shaped by glycan structure and availability, including a diverse array of polysaccharides presented through diet, host mucous secretions or shed epithelial cells, as well as microbial exopolysaccharides and capsules, all of which are undegradable by endogenous host enzymes [6]. Consistent with the importance of glycan metabolism in the adult host, gut microbiomes encode thousands of carbohydrate-active enzymes (CAZymes) and associated transport systems, which collectively enable polysaccharide uptake, depolymerization and fermentation into CO_2, H_2 and host-absorbable short-chain fatty acids (SCFAs) [7]. These metabolites can have both local effects, as nutrient for intestinal epithelium cells, or can be absorbed into the bloodstream and affect systemic functions [8]. While a large number of studies have applied genetic, biochemical and structural approaches to dissect the mechanisms that individual microbial cells use to degrade nutrients [6], the next outstanding challenge is to unravel the diverse activities and interactions that sequentially enable carbohydrate utilization dynamics in complex gut microbiomes. Here, we review recent studies that have led to the discovery of glycan-degrading activities and new CAZymes, with a focus on bacterial mechanisms described in single organisms and microbiomes, which have been facilitated by the application of multi-omic tools to identify activated genes and enzymatic pathways as well as microbial syntrophies in response to glycan cues.

Specialized polysaccharide utilization loci (PULs) enable nutrient utilization by gut-associated bacteria in a plethora of ecosystems

Microbiome processing of dietary and host-derived nutrients

Given that complex dietary and host-derived glycans contain several different monosaccharide constituents,

non-sugar residues, α-glycosidic or β-glycosidic linkages of various types, and substitutions (including acetylations, methylations, sulfation), depolymerization of these structures requires an array of linkage-specific endo-active and exo-active CAZymes (Figure 1).

CAZymes devoted to specific degradative functions are collected in the continuously updated CAZy database (http://www.cazy.org). Based on amino acid sequence similarity, CAZymes are currently (as per February 2022) grouped into 173 families of glycoside hydrolases (GHs), 42 families of polysaccharide lyases (PLs), and 20 families of carbohydrate esterases (CEs) [10].

Members of the Gram-negative Bacteroidetes and Gram-positive Firmicutes and Actinobacteria, which are ubiquitous and dominant phyla in gut microbial consortia of ruminants, monogastric animals and some insects, have evolved different strategies and vary widely in the number of glycans that they are capable of processing. Significant research has been focused on the study of polysaccharide degradation in the Bacteroidetes, and the huge number of diverse CAZymes (from 100 to over 300, with an average of 137.1 per genome [7]) that allows them to act as generalists, with broad glycan-utilization abilities, and to switch readily between different available substrates [11]. Bacteroidetes are known to arrange all the genes required for the recognition, uptake and depolymerization of a specific glycan into co-regulated gene clusters (operons) named polysaccharide utilization loci (PULs) [11]. Noteworthy, the term PUL was originally used to refer to glycan utilization gene clusters in the Gram-negative Bacteroidetes (gnPUL). As research has recently extended into members of the Firmicutes phylum, the PUL concept has been adapted to similar clusters of glycan degradation-related genes identified in Gram-positive bacteria (gpPUL) [12]. gnPULs are identified in Bacteroidetes genomes by the presence of one or more TonB-dependent receptor (SusC-homolog), a contiguous substrate-binding lipoprotein (SusD-homolog), a gene encoding one of three types of transcriptional regulators (Hybrid Two-Component System, ECF-σ/anti-σ regulator pairs or a SusR-homolog) and a suite of neighboring CAZymes tailored for the depolymerization of a particular substrate [11]. Generally, glycan decomposition in Bacteroidetes is initiated by a cell surface-located endo-active GH or PL. The resulting oligosaccharides are imported into the periplasm and further depolymerized by periplasmic GHs, PLs, CEs and sulfatases to yield disaccharides or monosaccharides, which are then internalized into the cytoplasm via inner membrane transporters and used as energy and carbon sources [11].

In contrast to the Bacteroidetes, the Firmicutes encode a lower proportional number of CAZymes and are specialized to target a few selected nutrients. Instead of the hallmark SusC-SusD pair that characterizes the gnPULs, gpPULs are defined and identified by the presence of closely flanked CAZyme genes, some form of a transcriptional regulator, and one of three classes of transporters: ATP-binding cassette (ABC) transporters, phosphoenolpyruvate: carbohydrate phosphotransferase system (PTS) transporters, or major facilitator superfamily (MFS) transporters [13]. In gpPUL-encoded glycan degrading systems, the target substrate is initially processed by extracellular CAZymes; the breakdown products are then recognized and bound by extracellular, high-affinity, solute binding proteins (SBPs), before being imported through ATP-dependent transport into the cytoplasm for intracellular decomposition. In Actinobacteria, studies have revealed the presence of gene clusters, including CAZyme-associated ABC transporters, similar to the gpPULs of the Firmicutes [12], but primarily associated with the utilization of oligosaccharides [14]. Discrete genetic loci encoding MFS, PTS or ABC transporter systems, a regulator, a limited number of exo-glycosidases and genes (such as kinases and isomerases) involved in metabolism of specific sugars have been described in Proteobacteria, a low abundance phylum in microbiomes. These systems are typically involved in monosaccharides and disaccharides catabolism by members of this phylum [15].

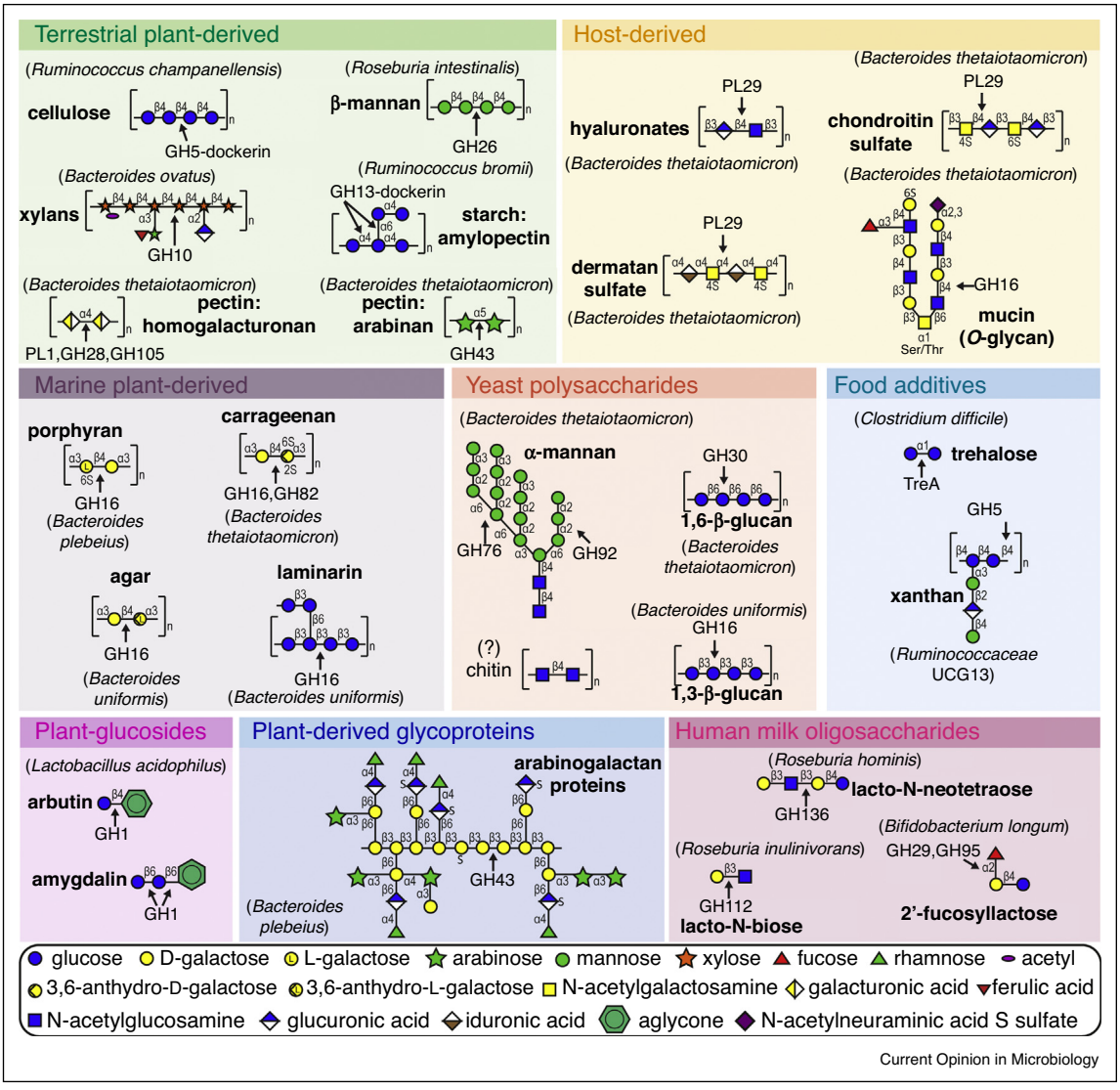
PULs are widespread in digestive ecosystems and have been shown to target virtually all of the known terrestrial plant-derived and yeast cell wall-derived glycans that are relevant to nutrition. Intriguingly, an increasing number of studies provide insights into the adaptation of microbiomes to constantly evolve their enzymatic utensils to metabolize glycans following the introduction and regular consumption of new food components. While detailed biochemically characterized gnPULs and gpPULs primarely derive from human gut studies, they are nonetheless ubiquitous in the guts of a vast variety of monogastrics (i.e. pigs, mice etc.), ruminants (cows, moose, reindeer) and insects (i.e. cockroaches, termites etc.) where they typically target starches, hemicelluloses and pectins (Table 1).

Degradation of atypical dietary glycans: seaweed-derived polysaccharides and food additives

Algal polysaccharides

Many cellulosic, hemicellulosic and pectic polysaccharide structures are surprisingly very common across different host-gut microbiome ecosystems, however, there exists some unique and subtle differences that are dependent on dietary and/or host sources. Glycans derived from seaweed are of particular interest for their chemical structures (including different sulfation patterns), distinct from those found in terrestrial plants, and for their variable consumption across different human populations. In landmark studies in 2010 and 2012, Hehemann and coworkers characterized a *Bacteroides plebeius* strain with the ability to degrade and grow on porphyran, as well as two

Figure 1



Source and schematic structures of host-derived glycans as well as glycans that are common in foods and feeds for human or animal consumption.
For each polysaccharide, the bacterium for which an exemplar utilization system has been identified and biochemically characterized is indicated in parentheses. Common endo-active CAZymes that initiate glycan degradation are shown. Monosaccharides are represented using symbol nomenclature for glycans [9]. Glycosidic bond linkages are shown while square brackets indicate repeating elements. Abbreviations: GH, glycoside hydrolase; PL, polysaccharide lyase; TreA, trehalase A. '?' indicates that such mechanism has not been discovered but may yet still exist in the human and/or animal gut.

gut *Bacteroides* that could grow on agarose and carrageenan, respectively [42,53]. Over the past decade, human gut bacteria have been found to contain PULs for catabolism of additional algal polysaccharides such as alginate [43,54]. In several cases, it appears that the enzymatic machinery for algal polyasccharide processing has been horizontally transferred from marine microbes into members of the gastrointestinal microbiome [6]. Human gut *Bacteroides* have also been shown to utilize 1,3-β-glucan (laminarin, Figure 1) from seaweed using specific GH families that are distinct from those involved in processing of terrestrial plant 1,3-β-glucans [31].

Sulfation is a prevalent modification of marine polysaccharides and arabinogalactan proteins (AGPs), which consist of up to 90% glycan, are no exception (Figure 1). A human gut *B. plebeius* was found to harbor a gnPUL for processing AGPs from seaweed [36]. Enzymatic depolymerization required the activity of a sulfatase that enables downstream CAZymes to access this substrate,

Table 1

Distribution of saccharolytic mechanisms that facilitate nutrient processing in human, rumen and insect gut ecosystems. Beside 'classical' gnPULs and gpPULs, other identified systems for glycan degradations in microbiomes include cellulosomes, amylosomes, the type 9 secretion system (T9SS, coupled [T9SS + PUL] or uncoupled [T9SS − PUL] to a PUL) and outer membrane vesicles (OMV)-mediated mechanisms (all described in the section 'Non-PUL mechanisms' below). Abbreviations: HMO, human milk oligosaccharides; MLG, mixed linkage β-glucans; RGII, rhamnogalacturonan II; AG, arabinogalactan; AGPs, arabinogalactan proteins; GAGs, glycosaminoglycans. A black circle indicates that the mechanism was determined based on biochemical data. A black triangle indicates that the mechanism is inferred from metagenomics data, with no confirmed phenotype and limited biochemical data available. An empty triangle indicates that the mechanism is inferred from metagenomics data, with no biochemical data; however, the bacterial isolate is available, and phenotype has been confirmed. Superscript ^{B} and ^{F} indicate bacterial and fungal cellulosome, respectively

Mechanism Host (fibers)	gnPUL	gpPUL	Cellulosome^{B}/ Amylosome^{B}	Cellulosome^{F}	T9SS + PUL	T9SS − PUL	OMVs	References
Human								
Cellulose			●					[16]
Starch	●	●	●					[17,18]
HMO	●	●	●				▲	[19–21]
α-mannan	●							[22]
β-glucan	●							[23]
Xyloglucan	●				▲			[24,25]
Xylans	●	●					▲	[21,26,27]
β-mannan	●	●						[28–30]
MLG	●							[31]
Fructans	●							[32]
Pectin	●							[33]
RGII	●							[34]
Arabinan/AG	●				▲			[25,35]
AGPs	●							[36]
O-glycans	●							[37]
N-glycans	●							[38]
GAGs	●							[39•]
Agarose	●							[40]
Porphyran	●							[41,42]
Laminarin	●							[31]
Alginate	●							[43]
Plant-glucosides	●							[44]
Rumen								
Cellulose	▲		●	●		▲	●	[45–49]
Starch	▲						●	[49]
Xylan	●		●	●		▲	●	[47]
β-mannan	●		●			▲	●	[47,50]
MLG	●		●			▲	●	[49]
Pectin	▲		●				●	[47]
Insect								
Cellulose	●/Δ							[51,52•]
Xylan	●							[51]
Xyloglucan	●							[51]
Starch	Δ							[52•]
Pectin	Δ				●			[52•]

leading the authors to conclude that this sulfatase provides *B. plebeius* privileged access to sulfated substrates including highly sulfated seaweed glycans [36].

A gut isolate of *Bacteroides thetaiotaomicron* that grows on lambda carrageenan (Figure 1) was recently characterized. Within a large gnPUL encoding many proteins with homology to functions in marine species, the *B. thetaiotaomicron* harbors a GH16 and two GH82 enzymes that hydrolyze lambda-carrageenan by an endo-mechanism but were unable to cleave kappa-carrageenans or iota-carrageenans, emphasizing that broad classes of polysaccharides still contain significant chemical variation with biological significance [55]. Several additional *Bacteroides* species were found to grow on lambda-carrageenan using partially homologous gnPULs that appear to reside on an integrative conjugative element that can be excised and circularized from the genome, then acquired by other microbial strains. A *Bacteroides xylanisolvens* strain that can utilize porphyran was found not to harbor a previously identified PUL that has apparently been mobilized into several different species; instead, it contains a large 96-gene region that is highly expressed during growth on porphyran, suggesting its involvement

in catabolizing this substrate. The first examples of Gram-positive gut microbes that degrade seaweed polysaccharides, a *Faecalicatena contorta* and a *Faecalicatena fissicatena*, grow on agarose and/or porphyran. These bacteria harbor gpPULs with genes encoding GH50 and GH86 enzymes that are able to hydrolyze agarose and/or porphyran. This latter study also demonstrated that the ability to use some seaweed polysaccharides, such as laminarin, is widespread in numerous human gastrointestinal microbes while utilization of other substrates is more sporadic [55].

Food additives

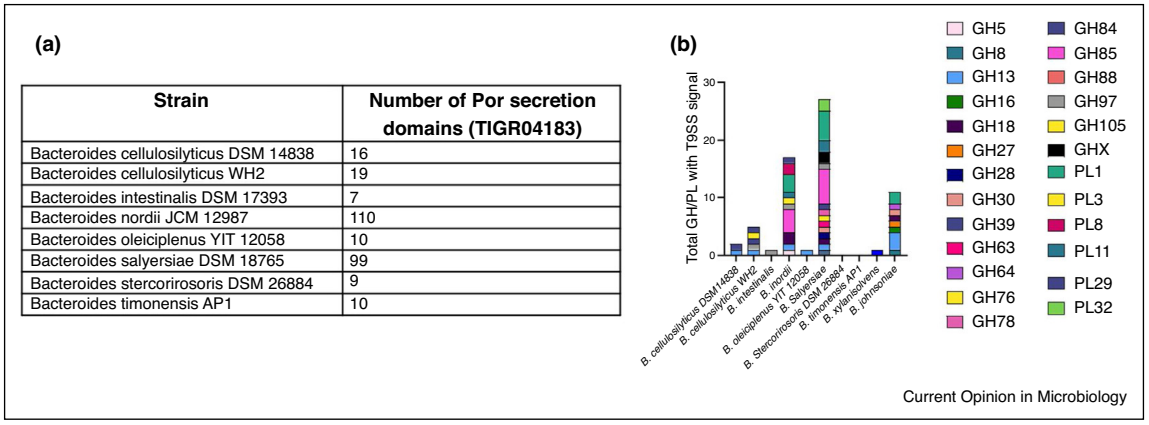
In contrast to seaweed polysaccharides that were introduced to the human diet several millennia ago, food additives such as xanthan gum and trehalose were included in processed foods only in the past few decades [56]. Through a combined culturing and multi-omics approach, a microbe from Ruminococcaceae uncultured genus 13 (*R.* UCG13) was discovered with the full enzymatic repertoire to saccharify the food stabilizer xanthan gum (Figure 1) [56]. In contrast to well-characterized pathways in environmental microbes that begin with removal of the terminal branched mannose before depolymerization, this organism uses a GH5 enzyme that hydrolyzes native xanthan gum to yield pentasaccharides. The released pentasaccharides can be consumed by the *R.* UCG13 itself as well as by a *Bacteroides intestinalis* strain with a distinct PUL. Both microbes appear to use a lyase and glucuronyl hydrolase to remove the terminal mannose and glucuronic acid from the pentasaccharide, followed by glycosyl hydrolases to saccharify the remaining trisaccharide structure [56]. Trehalose (Figure 1) was found to be the driver for the spread of specific epidemic lineages of *Clostridium difficile* that have acquired a PTS system for uptake of this disaccharide sugar and a trehalase (TreA) for its hydrolysis [57]. Together, these studies demonstrate that novel nutrients in the gut environment provides selective pressures for evolution of the microbiome to adapt and take advantage of the new nutrient niche.

Degradation of mucin glycans

Secreted mucus is a critical component of the gut defensive barrier and protects the host from physical and biological attacks. Mucin can consist up to 80% *O*-linked glycans and serves as a nutrient source for some commensal and pathogenic microbes. The primary constituent of secreted colonic mucus is a network of crosslinked, high molecular weight Mucin2 (Muc2) glycoproteins. These consists of a peptide backbone (with serine/threonine) decorated by over a hundred distinct glycan side chains [58]. A growing number of reports have shown that the colonic mucus is a major modulator of human health by increasing intestinal barrier function, thus maintaining stability of the intestinal environment, and decreasing inflammatory processes [59]. Intriguingly, Desai *et al.* demonstrated that deprivation of dietary polysaccharides promoted specific bacterial populations and their enzymes that target host mucin glycans as an energy source, which led to erosion of the colonic mucous layer and increased susceptibility to enteric pathogens [60]. The critical importance of this endogenous source of carbohydrates for the microbiota was further highlighted by a recent study that used mucin-derived *O*-glycans as effective prebiotics to mitigate microbiota perturbation and reduce the abundance of pathogenic *C. difficile* [61]. Because of the relevance of the mucus layer for maintaining health and in providing a nutrient niche for the microbiome, there is intense interest in understanding how different microbes degrade and consume this host-derived substrate. Multiple models exist for mucin degradation by gastrointestinal microbes that range from successive removal of individual terminal sugars to cleavage of larger mucin oligosaccharides or glycopeptides that are subsequently catabolized. Although they appear contradictory, these models are not mutually exclusive, with the metabolic pathway taken depending on the specific mucin substrate and microbe(s) involved. In an example of the 'exo-trimming' model, *Ruminococcus gnavus* uses an intramolecular *trans*-sialidase to cleave terminal sialic acid (aka *N*-acetylneuroaminic acid or Neu5Ac) from glycoproteins and release 2,7-anhydro-Neu5Ac, providing it with an advantage over other microbes that might also utilize mucus-derived sialic acids but cannot metabolize the anhydro form [62]. Bell *et al.* recently identified and characterized the transporter used by *R. gnavus* to import 2,7-anhydro-Neu5Ac as well as an oxidoreductase that regenerates Neu5Ac for a subsequent aldolase and entry into central metabolism [63]. Interestingly, some mucin-consuming microbes appear to produce sialidases that release these capping residues to access other parts of the *O*-glycan rather than to use the released sialic acids themselves [64].

B. thetaiotaomicron was shown to activate PULs associated with *O*-glycan metabolism in response to growth on free mucin oligosaccharides, but not their component monosaccharides. This suggests that this organism produces (or requires access to, by another bacterium) endo-acting enzymes that release intact mucin oligosaccharides that can be transported to the sites where they interact with transcriptional activators, often in the periplasm. Support for the 'endo-cleavage' model eventually came from the discovery and characterization of several GH16 enzymes from mucin-degrading gut microbes that can cleave the long oligosaccharide side-chains directly from mucin glycoprotein substrates [65]. These enzymes are able to tolerate some sulfation and fucosylation (albeit with unknown linkages) but are blocked by sialic acid, suggesting that for several species a hybrid model is appropriate in which both individual sugars and larger oligosaccharides are progressively removed to degrade the mucin glycans. Although most of this study was conducted with gastric or small intestinal mucus

Figure 2



Strain	Number of Por secretion domains (TIGR04183)
Bacteroides cellulosilyticus DSM 14838	16
Bacteroides cellulosilyticus WH2	19
Bacteroides intestinalis DSM 17393	7
Bacteroides nordii JCM 12987	110
Bacteroides oleiciplenus YIT 12058	10
Bacteroides salyersiae DSM 18765	99
Bacteroides stercorirosoris DSM 26884	9
Bacteroides timonensis AP1	10

Number of secretion domains that target proteins to the T9SS, and T9SS-associated CAZymes predicted in *Bacteroides* spp. **(a)** Predicted Type 9 secreted proteins from *Bacteroides* type species. Species containing a homolog of SprA (T9SS pore protein) were identified via Blast, and the Integrated Microbial genome database (img.jgi.doe.gov) was used to find proteins containing a TIGR04183 Por_Secre_Tail domain. **(b)** Predicted GH and PL families targeted for T9 secretion in *Bacteroides* spp. Proteins identified in (a) were classified into CAZy families using the CAZy database (Cazy.org) and DBcan (bcb.unl.edu/dbCAN2/index.php). The Bacteroidete *Flavobacterium johnsoniae* was included for comparison.

glycoproteins, the authors also used one GH16 to treat human colonic tissue obtained from patients and were able to detect released oligosaccharides; this provides some evidence that these enzymes cleave the backbone of mucin glycan side chains in an endo-acting fashion (endo-mucinases), although more detailed studies are required to confirm this. A subsequent study investigated how *B. thetaiotaomicron* (one of the microbes with a GH16 endo-mucinase) utilizes mucin *O*-glycans that were chemically released from colonic mucus, which are more heavily sulfated than the mucus glycans in earlier parts of the digestive tract [66•]. Highlighting the differences between gastric and colonic mucin glycans, the authors found two microbes that were capable of growth on gastric mucin glycans but not those from the colon. Surprisingly, while deletion of multiple sulfatases failed to reduce the ability of *B. thetaiotaomicron* to grow on sulfated colonic mucin glycans *in vitro*, a single sulfatase (BT1636) targeting galactose-3-sulfatate (3S-Gal) in a broad set of glycan contexts was found to be disproportionately important. Growth of a strain lacking BT1636 on colonic mucin glycans resulted in accumulation of sulfated oligosaccharides in the culture supernatant relative to a wildtype strain and had a competitive colonization defect in the mouse gut, further confirming the necessity of this enzyme for growth on this substrate. Together, the combined data from *B. thetaiotaomicron* mucin *O*-glycans-degradation suggest a model in which terminal sialic acids are removed first, providing access for GH16 endo-mucinases to release larger oligosaccharides. Some sulfated oligosaccharides are processed extracellularly by a sulfatase to make them accessible for further processing and catabolism. Shorter glycans on the mucin polypeptide may serve as recognition motifs for glycoproteases and additional GHs that complete the degradative process.

Non-PUL mechanisms

Type 9 secretion system (T9SS)

Many environmental Bacteroidetes use the Type 9 secretion system (T9SS) to secrete PUL-derived enzymes (reviewed in Ref. [11]). In particular, the T9SS permits the secretion of high molecular weight proteins, exemplified by the multi-modular ~160 kDa chitinase ChiA from the *Flavobacterium johnsoniae* chitin utilisation locus (ChiUL) [67,68]. Organisms lacking PULs, such as the cellulolytic *Cytophaga hutchinsonii* and *Sporocytophaga myxococcoides*, also use the T9SS to secrete multiple proteins [69], and the T9SS is key for some uncultivable putative fiber-degrading species found in the rumen [46]. Though T9SS-mediated secretion of CAZymes is well known within the wider Bacteroidetes phyla [11], T9SS was thought to be absent in most of the anaerobic gut *Bacteroides* species [70]. As more genome sequences have become available, it is clear that several *Bacteroides* sp. including *Bacteroides salyersiae*, *Bactetorides nordii* and *Bacteroides cellulosilyticus* possess the T9SS. Analyses of the occurrence of TIGR04183 (Por_Secre_tail) domain in *Bacteroides* reveal some species have over 100 proteins which are predicted to be secreted via the T9SS (Figure 2a). Several of these encode glycoside hydrolases and polysaccharide lyases — as many as 27 in *B. salyersiae* (Figure 2b), and the number of T9-secreted CAZymes is likely to increase as proteins of unknown function are studied.

In many cases the T9-secreted CAZymes are located in gnPULs associated with glycan degradation in

Bacteroides species. In *B. cellulosilyticus* WH2, three predicted T9SS substrates are shown to be upregulated during growth on xyloglucan (BACWH2_3125 and 3137, both proteins of unknown function) and arabinogalactan (BACWH2_2775, a carbohydrate binding module [CBM] belonging to the family 13 [CBM13]), indicating that T9 secreted proteins may play a key role in glycan degradation in the gut [25]. The predicted T9 secreted enzymes are in many cases larger than those predicted to be lipoproteins, suggesting that T9 secretion enables addition of accessory domains such as CBMs to surface located or secreted proteins that may be too large to secrete via other routes, perhaps advantaging *Bacteroides* sp. with T9SS.

In addition to detection of the T9SS in the human gut and rumen, a recent study reported the occurrence of all the necessary components of the T9SS in five glycan-degrading *Bacteroidetes* isolated from the alimentary canal of the cockroach *Periplaneta americana*. Interestingly, the *Bacteroides* sp. PAB214 of this study is one the first *Bacteroides* spp. genomes to display the complete set of genes for T9SS assembly [52•]. These *P. americana* associated *Bacteroidetes* contain multiple CAZyme-encoding genes, and are able to hydrolyze starch, cellulose and pectin, *in vitro*. Notably, some PLs (PL1, PL9, and PL11), rhamnogalacturonyl hydrolases (GH105), and β-galactosidases (GH2), of *Bacteroides* PAB214 and *Paludibacter* PAR221, isolated from the cockroaches, also possess the canonical C-terminal domain motif tag (PxGxYVV and KxxxK) necessary for T9SS translocation, suggesting these bacteria are able to use the T9SS for pectin catabolism [52•].

Cellulosomes and amylosomes

Cellulosomes are multi-enzyme complexes of CAZymes, mainly cellulases, that are tethered to cell-surface scaffoldin proteins via highly specific cohesin-dockerin interactions (recently reviewed in Ref. [45]). Cellulosomes were originally discovered in anaerobic bacteria but are also used by certain anaerobic fungi, where they function in much the same way, and play a vital role in biomass degradation in diverse ecosystems [71]. Within the rumen of herbivores, both fungal and bacterial cellulosomes are prominent (Table 1). For example, the renowned cellulose degraders *Ruminococcus flavefaciens* and *Ruminococcus albus* utilize cellulosomes to contribute to plant biomass degradation [72,73] and are frequently detected in metaomics studies [49,50]. The distribution of fungal cellulosomes in the rumen is not as well understood, but emerging omics studies [4•,74•] are highlighting their key role in nutrient processing. Cellulose is not generally metabolized by the human gut Bacteroidetes, while studies have shown that the Firmicute *Ruminococcus champanellensis* can digest microcrystalline cellulose [16]. Similarly, the amylosome of the human gut resident *Ruminococcus bromii* has been characterized; this species produces 15 distinct GH13 starch-degrading enzymes and may be largely responsible for the processing of resistant starches that escape hydrolysis by human enzymes and acids [18].

Outer membrane vesicles

Outer membrane vesicles (OMVs) are blebbed from the bacterial cell membrane and have been shown to serve as vehicles of CAZymes that target plant polysaccharides within the rumen and the human gut. OMVs-mediated polysaccharide degrading mechanisms have been reported in the rumen bacterium *Fibrobacter succinogenes* during *in vitro* growth on cellulose [47]. Using a proteomic and enzymatic approach to characterize their content, Arntzen *et al.* showed that these OMVs are enriched in GHs and multiprotein complexes, allowing *F. succinogenes* to effectively bind and to degrade plant cellulose, hemicelluloses and pectin [47]. In a recent study, OMVs were also observed *in vivo* in a sheep model colonized with *F. succinogenes* [75]. Also, human-gut derived bacteria such as *Bacteroides fragilis* and *B. thetaiotaomicron* produce OMVs loaded with hydrolytic enzymes that are important in polysaccharide degradation [21].

Microbiomes and their mechanisms interact to degrade nutrients

Nutrient competition and sharing among members of the human gut microbiota

A wealth of recent research has identified a network of intricate interplays between the different saccharolytic mechanisms that glycan degraders encode within microbiomes. Importantly, data indicates that a few generally occurring 'keystone' species initiate the breakdown of more recalcitrant, full-length, polymers and release oligosaccharides and monosaccharides in the environment. These organisms play unique and essential roles in microbiomes, and their removal can cause dramatic changes in the structure and metabolic functions of the microbial community [76]. Prominent examples of keystone organisms in the human gut microbiota are the resistant starch degrader *R. bromii* [77], the mucin degrader *Akkermansia muciniphila* [78], and the arabinogalactan degrader *B. thetaiotaomicron* [35]. Breakdown products generated by the activity of keystone species are taken up by secondary degraders for their own benefit, with the establishment of cross-feeding interactions. 'Nutrient-sharing' has been observed between species in the Firmicutes and Bacteroidetes phyla [6]. As an example, a recent investigation showed that both the primary β-mannan degraders *Roseburia intestinalis* (gpPUL) and *Bacteroides ovatus* (gnPUL) supported the growth of *Faecalibacterium prausnitizii* that, despite encoding a β-mannan gpPUL, lacks an extracellular endo-mannanase, and thus depends on β-mannooligosaccharides (β-MOS) generated by another species [79]. Conversely, 'selfish' glycan catabolism and competitive interactions are established when the mechanism for polysaccharide utilization by a primary degrader prevents the loss of free oligosaccharide or simple sugars into the

extracellular milieu. For examples, 'selfishness' has been shown during α-mannan processing by *B. thetaiotaomicron* [22], where the oligosaccharides produced by surface endo-active enzymes are immediately captured and transported into the cell, such that they are not available to other strains of *Bacteroides* [22]. Competition mechanisms involving two or more primary glycan degraders in overlapping glycan niches have also been described, wherein the species with the most efficient glycan utilization system has a greater growth advantage and will outcompete the other. Such a phenomenon has recently been described between *B. ovatus* competing with *R. intestinalis or Bifidobacterium animalis* subsp. *lactis* B1-04 in co-culture on β-mannan. *B. ovatus* was out-competed by the two Gram-positive bacteria; the competitive advantage was likely conferred by their gpPUL-associated SBP-ABC transporter systems for high-affinity binding and import of β-MOS generated thought the activity of extracellular endo-mannanases [28,29].

Trans-kingdom cooperation in the rumen microbiota

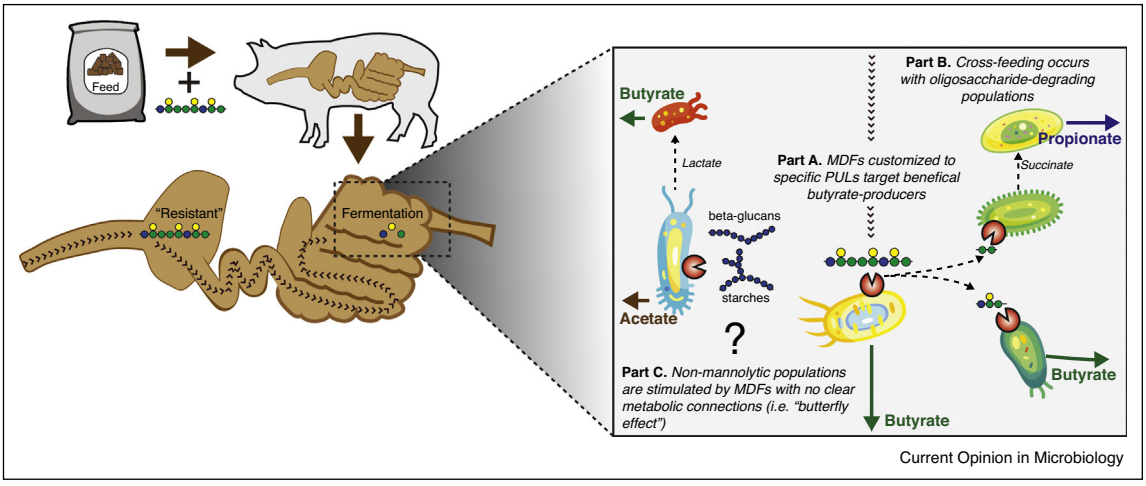
Given the importance of ruminant livestock systems to produce edible food for mankind, extensive research has been conducted to understand catabolic activities enabling the rumen microbial community to efficiently digest their plant feed. This knowledge is critical to develop strategies for improving animal health and productivity as well as mitigate negative impacts of the livestock industry, such as greenhouse gas emissions (e.g. methane). A multitude of studies has shown that fungi, protozoa and bacteria all process dietary fiber using a variety of mechanisms including cellulosomes, PULs, T9SS and OMVs (see review Ref. [80]). *In vivo* competitive interactions between predominant cellulolytic bacterial species found in the rumen (*F. succinogenes*, *R. albus*, and *R. flavefaciens*) have been recently elucidated in a study that used a gnotobiotic sheep model [75]. Here, although the bacteria showed different but equally efficient systems for lignocellulose breakdown, *F. succinogenes* was gradually outcompeted by the two ruminococci, with concomitant changes in the fermentation end products. Transcriptomics revealed that *F. succinogenes* exhibited high expression of genes predicted to be involved in the production of OMVs and CAZymes for cellulose and hemicellulose decomposition while *R. albus* and *R. flavefaciens* relied on type IV pili and CAZymes harboring either a CBM37 or a dockerin (for cellulosome assembly) to degrade the same substrates [75].

In addition to bacterial interactions, recent studies applying multi-omic techniques have revealed that the mechanisms governing nutrient processing in these ecosystems include viral and fungal activities. Hagen *et al.* showed that anaerobic fungi are key players in the degradation of recalcitrant biomass in cows, primarily through the activity of cellulosomal enzymes (including enzymes belonging to the GH families GH5, GH6, GH8, and GH48) while bacterial populations contribute mostly with CAZymes associated with the degradation of more readily degradable carbohydrates such as starch and hemicelluloses [4•]. Similarly, Peng *et al.* enriched microbial consortia from the goat rumen on several lignocellulosic substrates, and showed that biomass decomposition is achieved through complementary hydrolytic strategies deployed by anaerobic fungi and bacteria [74•]. Compared to bacterial and eukaryotic populations much less is known regarding the 'influence' of the virome on nutrient processing, however as the resolution of omics techniques improve, a clearer picture is emerging. Solden *et al.* identified hundreds of viral populations within the rumen of Alaskan moose and suggested that virus-mediated bacterial lysis could contribute to the release of microbial CAZymes and enhance carbohydrate breakdown within the rumen [50].

Linking biochemical mechanisms to dietary intervention: microbiota-directed fibers for precise manipulation of gut microbiomes

Fundamental research on saccharolytic mechanisms and the rapid advancement of next-generation omics-technologies are gradually allowing the global exploration of food-networks that enable nutrients utilization in a real world 'community-context' [81]. Understanding of these networks is crucial to facilitate the development of microbiota-directed fibers (MDFs) that selectively target and enhance the abundance of beneficial gut microbes and their metabolic functions relative to health. Indeed, fibers with chemical structures that align with enzymatic capabilities of specific bacteria could be used to obtain predictable changes in microbiota composition or even implant engineered bacteria independent of the competitive pressures of the environment for nutrient acquisition. Despite its potential, examples of MDF applications are limited and have only been demonstrated on selected fibers. In mice, administration of porphyran was used for a targeted, predictable, and dose-dependent increase in a *B. ovatus* population harboring a rare cluster for utilization of this glycan [41], while β-mannan promoted predictable expansion of mannolytic *Bacteroides*, *Roseburia* and *Marvinbryantia* species [28]. A recent study integrated metagenomics and metaproteomics approaches to determine the effect of a highly tailored β-mannan fiber (spruce-derived acetylated galactoglucomannan, AcGGM) on the weaning pig colon microbiota [82••] (Figure 3). This AcGGM MDF was tailored towards the enrichment of beneficial microbes and, indeed, activated a metabolic response in specific *Roseburia* and *Faecalibacterium* populations, both renowned butyrate-producing gut commensals, equipped with the necessary enzyme systems to metabolize AcGGM. Notably, MDF-inclusion resulted in the co-called 'butterfly effect', which implies changes in the gut microbiome that are not directly related to breakdown of this specific MDF (see Figure 3 and Ref. [82••]).

Figure 3



Highly tailored MDFs can be designed to be resistant to host digestion but match unique enzymatic capabilities of beneficial target organisms in gut microbiomes (Part A). However, dietary interventions can also inadvertently stimulate other populations incapable of utilizing the MDFs (with no evidence of being directly related to the utilization of the tested fiber; Part B–C) that can likely take advantage of underlying availabilities of hydrolysis and/or fermentation intermediates (Part C).

In human trials, three structurally different type-IV resistant starch-based MDFs caused highly specific effects on gut microbiota diversity and composition. These MDF-induced changes led to selective enrichments of a few bacterial taxa that possess the enzymatic toolbox to metabolize the respective substrates and changed microbiota functions, with directed shifts in SCFA output toward either butyrate or propionate [83••]. In a recent study with gnotobiotic mice colonized with a 15-member artificial community and fed a diet containing 34 food-grade fibers, a combined quantitative proteomic and genome-wide transposon mutagenesis approach showed that different dietary fibers selectively support the expansion of targeted *Bacteroides in vivo* and induce the expression of the appropriate gnPUL only when required [84••]. In a follow-up study that tested the translatability of results obtained in gnotobiotic mice, snack prototypes containing one, two or four fiber (pea, orange, barley, inulin) blends were subsequently tested in controlled-diet studies with pilot cohorts of obese or overweight individuals [85••]. Using machine-learning and multi-omics, the study showed that the four-fiber diet produced significant increases in Bacteroidetes species, induced a greater spectrum of glycan-metabolizing enzymes (some of which were not directly linked to catabolism of glycans in the snacks, aka 'butterfly effect') in the gut microbiome and increased expression of genes inversely associated with obesity [85••].

Overall, these studies open the path for precision nutrition, whereby designer MDFs, acting as a prebiotic, could be tailored to selectively target beneficial microbes at genera/strain level to establish, restore, and/or sustain a healthy gut microbiome. However, it must be reiterated that in order to establish a predictable functional connection between a given MDF structure and specific microbial populations that encode for its degradation, the unequivocal characterization of all the stereochemical information within a given glycan is critical and still remains a challenge in the field of glycomics. In recent years, however, a combination of innovative ion activation methods, commercialization of state-of-the-art ion mobility–mass spectrometry instruments, the introduction of gas-phase ion spectroscopy to the field, as well as in advances in nuclear magnetic resonance and in computational chemistry have provided many fundamental insights into the structural complexity of these biomolecules at a rapid pace. It is expected that further significant progress will be made in analytical techniques to enhance the glycomics toolbox in the near future [86,87].

Conclusions and future directions

Nutrient catabolism in gut microbiomes is a 'team effort' determined by interplays between primary degraders and cross-feeders. Our current knowledge of the functions of microbial populations involved in glycan utilization has mainly been gained from isolated microorganisms. Strikingly, a wealth of research has revealed that these microbes have adapted to consume both common and 'new' glycans introduced through diet using preexisting or constantly evolving enzymatic toolboxes. Recent advancements in culture-independent meta-omic technologies have provided new perspectives on understanding nutrient metabolism beyond individual functions and highlight the existence of a wide range of interaction networks as well as underexplored non-fiber-driven

effects (also referred as 'butterfly effects') involving a multitude of microbial populations. Still, we know very little of the contributions made to nutrient catabolism by less understood members of the gut microbiota, such as viruses, fungi, protozoa and archaea. Uncovering these currently less accessible microbiota and exploring their diversity and functional role will be crucial for visualizing inter-organismal interactions that drive the overall nutrient processing at the greater microbiome level. Future efforts to generate a comprehensive functional understanding of the microbiome will require both metagenomics and culture efforts to expand the number of characterized non-bacterial members, and for the generation of more complete genome sequence databases to which functional omics datasets can be mapped against. Optimal integration of biochemical and functional metaomic data as well as the development of necessary tools to analyze the huge amounts of varying data types will be key to elucidating the networks that exist between all the 'microbial pieces of the gut puzzle'. Given the pace of discovery and the technology advancements in the last decade, we anticipate that investigations of nutrient metabolism by gut microbes will continue at a rapid pace to yield a deeper understanding of the composition, ecology and functioning of these ecosystems and will create opportunities for modulating gut microbiomes to promote or restore host health via targeted delivery of fibers.

Conflict of interest statement

Nothing declared.

Acknowledgements

PBP and SLLR acknowledge financial support for their work through The Research Council of Norway (Project no. 250479 and 300846), the European Union's Horizon 2020 research and innovation programme under the ERA-Net Cofund project BlueBio (grant agreement no. 311913), and the Novo Nordisk Foundation (Project no. 0054575 - SuPAcow). Support for LSM is provided by the Wallenberg Wood Science Centre and by funds awarded by the Swedish Research Council Vetenskapsrådet (project 2017-04906).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Zheng D, Liwinski T, Elinav E: **Interaction between microbiota and immunity in health and disease**. *Cell Res* 2020, **30**:492-506.

2. Fan Y, Pedersen O: **Gut microbiota in human metabolic health and disease**. *Nat Rev Microbiol* 2021, **19**:55-71.

3. • Vera-Ponce de Leon A, Jahnes BC, Otero-Bravo A, Sabree ZL: **Microbiota perturbation or elimination can inhibit normal development and elicit a starvation-like response in an omnivorous model invertebrate**. *mSystems* 2021, **6**:e0080221

In this study, the authors show that germ-free rearing of a cockroach model resulted in growth defects and severely disrupted gene networks that regulate development, thus highlighting the importance of the gut microbiota in insect nutrition and other physiological processes.

4. • Hagen LH, Brooke CG, Shaw CA, Norbeck AD, Piao H, Arntzen MO, Olson HM, Copeland A, Isern N, Shukla A *et al.*: **Proteome specialization of anaerobic fungi during ruminal degradation of recalcitrant plant fiber**. *ISME J* 2021, **15**:421-434

This study leveraged a genome-centric metaproteomic and metatranscriptomic approach to show the contribution of rumen eukarya and bacteria (*in situ*, in cannulated cows) to plant biomass degradation. Based on the results, the authors reconstructed active metabolic pathways that lead to production of host-absorbable short-chain fatty acids.

5. Collado MC, Cernada M, Bauerl C, Vento M, Perez-Martinez G: **Microbial ecology and host-microbiota interactions during early life stages**. *Gut Microbes* 2012, **3**:352-365.

6. Glowacki RWP, Martens EC: **If you eat it, or secrete it, they will grow: the expanding list of nutrients utilized by human gut bacteria**. *J Bacteriol* 2020, **203**:e00481-20 http://dx.doi.org/10.1128/JB.00481-20.

7. El Kaoutari A, Armougom F, Gordon JI, Raoult D, Henrissat B: **The abundance and variety of carbohydrate-active enzymes in the human gut microbiota**. *Nat Rev Microbiol* 2013, **11**:497-504.

8. Louis P, Flint HJ: **Formation of propionate and butyrate by the human colonic microbiota**. *Environ Microbiol* 2017, **19**:29-41.

9. Neelamegham S, Aoki-Kinoshita K, Bolton E, Frank M, Lisacek F, Lutteke T, O'Boyle N, Packer NH, Stanley P, Toukach P *et al.*: **Updates to the symbol nomenclature for glycans guidelines**. *Glycobiology* 2019, **29**:620-624.

10. Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B: **The carbohydrate-active enzymes database (CAZy) in 2013**. *Nucleic Acids Res* 2014, **42**:D490-495.

11. McKee LS, La Rosa SL, Westereng B, Eijsink VG, Pope PB, Larsbrink J: **Polysaccharide degradation by the Bacteroidetes: mechanisms and nomenclature**. *Environ Microbiol Rep* 2021, **13**:559-581.

12. Sheridan PO, Martin JC, Lawley TD, Browne HP, Harris HMB, Bernalier-Donadille A, Duncan SH, O'Toole PW, Scott KP, Flint HJ: **Polysaccharide utilization loci and nutritional specialization in a dominant group of butyrate-producing human colonic Firmicutes**. *Microb Genom* 2016, **2**:e000043.

13. Cockburn DW, Koropatkin NM: **Polysaccharide degradation by the intestinal microbiota and its influence on human health and disease**. *J Mol Biol* 2016, **428**:3230-3252.

14. Fushinobu S, Abou Hachem M: **Structure and evolution of the bifidobacterial carbohydrate metabolism proteins and enzymes**. *Biochem Soc Trans* 2021, **49**:563-578.

15. Ndeh D, Gilbert HJ: **Biochemistry of complex glycan depolymerisation by the human gut microbiota**. *FEMS Microbiol Rev* 2018, **42**:146-164.

16. Ben David Y, Dassa B, Borovok I, Lamed R, Koropatkin NM, Martens EC, White BA, Bernalier-Donadille A, Duncan SH, Flint HJ *et al.*: **Ruminococcal cellulosome systems from rumen to human**. *Environ Microbiol* 2015, **17**:3407-3426.

17. Cerqueira FM, Photenhauer AL, Pollet RM, Brown HA, Koropatkin NM: **Starch digestion by gut bacteria: crowdsourcing for carbs**. *Trends Microbiol* 2020, **28**:95-108.

18. Ze X, Ben David Y, Laverde-Gomez JA, Dassa B, Sheridan PO, Duncan SH, Louis P, Henrissat B, Juge N, Koropatkin NM *et al.*: **Unique organization of extracellular amylases into amylosomes in the resistant starch-utilizing human colonic Firmicutes bacterium *Ruminococcus bromii***. *mBio* 2015, **6** e01058-01015.

19. Pichler MJ, Yamada C, Shuoker B, Alvarez-Silva C, Gotoh A, Leth ML, Schoof E, Katoh T, Sakanaka M, Katayama T *et al.*: **Butyrate producing colonic *Clostridiales* metabolise human milk oligosaccharides and cross feed on mucin via conserved pathways**. *Nat Commun* 2020, **11**:3285.

20. Kujawska M, La Rosa SL, Roger LC, Pope PB, Hoyles L, McCartney AL, Hall LJ: **Succession of *Bifidobacterium longum* strains in response to a changing early life nutritional environment reveals dietary substrate adaptations**. *iScience* 2020, **23**:101368.

21. Elhenawy W, Debelyy MO, Feldman MF: **Preferential packing of acidic glycosidases and proteases into *Bacteroides* outer membrane vesicles**. *mBio* 2014, **5**:e00909-e00914.

22. Cuskin F, Lowe EC, Temple MJ, Zhu Y, Cameron E, Pudlo NA, Porter NT, Urs K, Thompson AJ, Cartmell A *et al.*: **Human gut Bacteroidetes can utilize yeast mannan through a selfish mechanism**. *Nature* 2015, **517**:165-169.

23. Temple MJ, Cuskin F, Basle A, Hickey N, Speciale G, Williams SJ, Gilbert HJ, Lowe EC: **A Bacteroidetes locus dedicated to fungal 1,6-beta-glucan degradation: unique substrate conformation drives specificity of the key endo-1,6-beta-glucanase**. *J Biol Chem* 2017, **292**:10639-10650.

24. Larsbrink J, Rogers TE, Hemsworth GR, McKee LS, Tauzin AS, Spadiut O, Klinter S, Pudlo NA, Urs K, Koropatkin NM *et al.*: **A discrete genetic locus confers xyloglucan metabolism in select human gut Bacteroidetes**. *Nature* 2014, **506**:498-502.

25. McNulty NP, Wu M, Erickson AR, Pan C, Erickson BK, Martens EC, Pudlo NA, Muegge BD, Henrissat B, Hettich RL *et al.*: **Effects of diet on resource utilization by a model human gut microbiota containing *Bacteroides cellulosilyticus* WH2, a symbiont with an extensive glycobiome**. *PLoS Biol* 2013, **11**:e1001637.

26. Rogowski A, Briggs JA, Mortimer JC, Tryfona T, Terrapon N, Lowe EC, Basle A, Morland C, Day AM, Zheng H *et al.*: **Glycan complexity dictates microbial resource allocation in the large intestine**. *Nat Commun* 2015, **6**:7481.

27. Leth ML, Ejby M, Workman C, Ewald DA, Pedersen SS, Sternberg C, Bahl MI, Licht TR, Aachmann FL, Westereng B *et al.*: **Differential bacterial capture and transport preferences facilitate co-growth on dietary xylan in the human gut**. *Nat Microbiol* 2018, **3**:570-580.

28. La Rosa SL, Leth ML, Michalak L, Hansen ME, Pudlo NA, Glowacki R, Pereira G, Workman CT, Arntzen MO, Pope PB *et al.*: **The human gut Firmicute *Roseburia intestinalis* is a primary degrader of dietary beta-mannans**. *Nat Commun* 2019, **10**:905.

29. Ejby M, Guskov A, Pichler MJ, Zanten GC, Schoof E, Saburi W, Slotboom DJ, Abou Hachem M: **Two binding proteins of the ABC transporter that confers growth of *Bifidobacterium animalis* subsp. *lactis* ATCC27673 on beta-mannan possess distinct manno-oligosaccharide-binding profiles**. *Mol Microbiol* 2019, **112**:114-130.

30. Bagenholm V, Wiemann M, Reddy SK, Bhattacharya A, Rosengren A, Logan DT, Stalbrand H: **A surface-exposed GH26 beta-mannanase from *Bacteroides ovatus*: structure, role, and phylogenetic analysis of BoMan26B**. *J Biol Chem* 2019, **294**:9100-9117.

31. Dejean G, Tamura K, Cabrera A, Jain N, Pudlo NA, Pereira G, Viborg AH, Van Petegem F, Martens EC, Brumer H: **Synergy between cell surface glycosidases and glycan-binding proteins dictates the utilization of specific beta(1,3)-glucans by human gut *Bacteroides***. *mBio* 2020, **11**.

32. Joglekar P, Sonnenburg ED, Higginbottom SK, Earle KA, Morland C, Shapiro-Ward S, Bolam DN, Sonnenburg JL: **Genetic variation of the SusC/SusD homologs from a polysaccharide utilization locus underlies divergent fructan specificities and functional adaptation in *Bacteroides thetaiotaomicron* strains**. *mSphere* 2018, **3**.

33. Ndeh D, Rogowski A, Cartmell A, Luis AS, Basle A, Gray J, Venditto I, Briggs J, Zhang X, Labourel A *et al.*: **Complex pectin metabolism by gut bacteria reveals novel catalytic functions**. *Nature* 2017, **544**:65-70.

34. Luis AS, Briggs J, Zhang X, Farnell B, Ndeh D, Labourel A, Basle A, Cartmell A, Terrapon N, Stott K *et al.*: **Dietary pectic glycans are degraded by coordinated enzyme pathways in human colonic *Bacteroides***. *Nat Microbiol* 2018, **3**:210-219.

35. Cartmell A, Munoz-Munoz J, Briggs JA, Ndeh DA, Lowe EC, Basle A, Terrapon N, Stott K, Heunis T, Gray J *et al.*: **A surface endogalactanase in *Bacteroides thetaiotaomicron* confers keystone status for arabinogalactan degradation**. *Nat Microbiol* 2018, **3**:1314-1326.

36. Munoz-Munoz J, Ndeh D, Fernandez-Julia P, Walton G, Henrissat B, Gilbert HJ: **Sulfation of arabinogalactan proteins confers privileged nutrient status to *Bacteroides plebeius***. *mBio* 2021, **12**:e0136821.

37. Gonzalez-Morelo KJ, Vega-Sagardia M, Garrido D: **Molecular insights into *O*-linked glycan utilization by gut microbes**. *Front Microbiol* 2020, **11**:591568.

38. Briliute J, Urbanowicz PA, Luis AS, Basle A, Paterson N, Rebello O, Hendel J, Ndeh DA, Lowe EC, Martens EC *et al.*: **Complex *N*-glycan breakdown by gut *Bacteroides* involves an extensive enzymatic apparatus encoded by multiple co-regulated genetic loci**. *Nat Microbiol* 2019, **4**:1571-1581.

39. • Ndeh D, Basle A, Strahl H, Yates EA, McClurgg UL, Henrissat B, Terrapon N, Cartmell A: **Metabolism of multiple glycosaminoglycans by *Bacteroides thetaiotaomicron* is orchestrated by a versatile core genetic locus**. *Nat Commun* 2020, **11**:646

The authors of this study conducted biochemical, genetic and structural analyses to characterize the machinery enabling glycosaminoglycan metabolism in the *Bacteroides thetaiotaomicron*. Remarkably, the study reports the discovery of two surface glycan binding proteins which facilitate import of chondroitin sulfate, dermatan sulfate and hyaluronic acid into the periplasm.

40. Pluvinage B, Grondin JM, Amundsen C, Klassen L, Moote PE, Xiao Y, Thomas D, Pudlo NA, Anele A, Martens EC *et al.*: **Molecular basis of an agarose metabolic pathway acquired by a human intestinal symbiont**. *Nat Commun* 2018, **9**:1043.

41. Shepherd ES, DeLoache WC, Pruss KM, Whitaker WR, Sonnenburg JL: **An exclusive metabolic niche enables strain engraftment in the gut microbiota**. *Nature* 2018, **557**:434-438.

42. Hehemann JH, Kelly AG, Pudlo NA, Martens EC, Boraston AB: **Bacteria of the human gut microbiome catabolize red seaweed glycans with carbohydrate-active enzyme updates from extrinsic microbes**. *Proc Natl Acad Sci U S A* 2012, **109**:19786-19791.

43. Thomas F, Barbeyron T, Tonon T, Genicot S, Czjzek M, Michel G: **Characterization of the first alginolytic operons in a marine bacterium: from their emergence in marine Flavobacteriia to their independent transfers to marine Proteobacteria and human gut *Bacteroides***. *Environ Microbiol* 2012, **14**:2379-2394.

44. Theilmann MC, Goh YJ, Nielsen KF, Klaenhammer TR, Barrangou R, Abou Hachem M: ***Lactobacillus acidophilus* metabolizes dietary plant glucosides and externalizes their bioactive phytochemicals**. *mBio* 2017, **8**.

45. Alves VD, Fontes C, Bule P: **Cellulosomes: highly efficient cellulolytic complexes**. *Subcell Biochem* 2021, **96**:323-354.

46. Naas AE, Solden LM, Norbeck AD, Brewer H, Hagen LH, Heggenes IM, McHardy AC, Mackie RI, Pasa-Tolic L, Arntzen MO *et al.*: **"*Candidatus Paraporphyromonas polyenzymogenes*" encodes multi-modular cellulases linked to the type IX secretion system**. *Microbiome* 2018, **6**:44.

47. Arntzen MO, Varnai A, Mackie RI, Eijsink VGH, Pope PB: **Outer membrane vesicles from *Fibrobacter succinogenes* S85 contain an array of carbohydrate-active enzymes with versatile polysaccharide-degrading capacity**. *Environ Microbiol* 2017, **19**:2701-2714.

48. Naas AE, Mackenzie AK, Mravec J, Schuckel J, Willats WG, Eijsink VG, Pope PB: **Do rumen Bacteroidetes utilize an alternative mechanism for cellulose degradation?** *mBio* 2014, **5**:e01401-e01414.

49. Stewart RD, Auffret MD, Warr A, Walker AW, Roehe R, Watson M: **Compendium of 4,941 rumen metagenome-assembled genomes for rumen microbiome biology and enzyme discovery**. *Nat Biotechnol* 2019, **37**:953-961.

50. Solden LM, Naas AE, Roux S, Daly RA, Collins WB, Nicora CD, Purvine SO, Hoyt DW, Schuckel J, Jorgensen B *et al.*: **Interspecies cross-feeding orchestrates carbon degradation in the rumen ecosystem**. *Nat Microbiol* 2018, **3**:1274-1284.

51. Liu N, Li H, Chevrette MG, Zhang L, Cao L, Zhou H, Zhou X, Zhou Z, Pope PB, Currie CR *et al.*: **Functional metagenomics reveals abundant polysaccharide-degrading gene clusters and cellobiose utilization pathways within gut microbiota of a wood-feeding higher termite**. *ISME J* 2019, **13**:104-117.

52. • Vera-Ponce de Leon A, Jahnes BC, Duan J, Camuy-Velez LA, Sabree ZL: **Cultivable, host-specific bacteroidetes symbionts exhibit diverse polysaccharolytic strategies**. *Appl Environ Microbiol* 2020, **86**

The authors of this study showed the presence of microbes capable of breaking down dietary fibers available in the alimentary canal of the omnivorous American cockroach, *Periplaneta americana*. Degradation was confirmed by isolation of the microbes and plate-based assays based on genome-inferred polysaccharide hydrolytic activity.

53. Hehemann JH, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G: **Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota**. *Nature* 2010, **464**:908-912.

54. Mathieu S, Touvrey-Loiodice M, Poulet L, Drouillard S, Vincentelli R, Henrissat B, Skjak-Braek G, Helbert W: **Ancient acquisition of "alginate utilization loci" by human gut microbiota**. *Sci Rep* 2018, **8**:8075.

55. Pudlo NA, Pereira GV, Parnami J, Cid M, Markert S, Tingley JP, Unfried F, Ali A, Varghese NJ, Kim KS *et al.*: **Diverse events have transferred genes for edible seaweed digestion from marine to human gut bacteria**. *Cell Host Microbe* 2022 http://dx.doi.org/10.1016/j.chom.2022.02.001. S1931-3128(22)00087-7.

56. Ostrowski MP, La Rosa SL, Kunath BJ, Robertson A, Pereira G, Hagen LH, Varghese NJ, Qiu L, Yao T, Flint G *et al.*: **The food additive xanthan gum drives adaptation of the human gut microbiota**. *bioRxiv* 2021 http://dx.doi.org/10.1101/2021.06.02.446819. 2021.2006.2002.446819.

57. Collins J, Robinson C, Danhof H, Knetsch CW, van Leeuwen HC, Lawley TD, Auchtung JM, Britton RA: **Dietary trehalose enhances virulence of epidemic *Clostridium difficile***. *Nature* 2018, **553**:291-294.

58. Larsson JM, Karlsson H, Sjovall H, Hansson GC: **A complex, but uniform *O*-glycosylation of the human MUC2 mucin from colonic biopsies analyzed by nanoLC/MSn**. *Glycobiology* 2009, **19**:756-766.

59. Belzer C: **Nutritional strategies for mucosal health: the interplay between microbes and mucin glycans**. *Trends Microbiol* 2022, **30**:13-21.

60. Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, Pudlo NA, Kitamoto S, Terrapon N, Muller A *et al.*: **A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility**. *Cell* 2016, **167**:1339-1353 e1321.

61. Pruss KM, Marcobal A, Southwick AM, Dahan D, Smits SA, Ferreyra JA, Higginbottom SK, Sonnenburg ED, Kashyap PC, Choudhury B *et al.*: **Mucin-derived *O*-glycans supplemented to diet mitigate diverse microbiota perturbations**. *ISME J* 2021, **15**:577-591.

62. Tailford LE, Owen CD, Walshaw J, Crost EH, Hardy-Goddard J, Le Gall G, de Vos WM, Taylor GL, Juge N: **Discovery of intramolecular trans-sialidases in human gut microbiota suggests novel mechanisms of mucosal adaptation**. *Nat Commun* 2015, **6**:7624.

63. Bell A, Brunt J, Crost E, Vaux L, Nepravishta R, Owen CD, Latousakis D, Xiao A, Li W, Chen X *et al.*: **Elucidation of a sialic acid metabolism pathway in mucus-foraging *Ruminococcus gnavus* unravels mechanisms of bacterial adaptation to the gut**. *Nat Microbiol* 2019, **4**:2393-2404.

64. Ng KM, Ferreyra JA, Higginbottom SK, Lynch JB, Kashyap PC, Gopinath S, Naidu N, Choudhury B, Weimer BC, Monack DM *et al.*: **Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens**. *Nature* 2013, **502**:96-99.

65. Crouch LI, Liberato MV, Urbanowicz PA, Basle A, Lamb CA, Stewart CJ, Cooke K, Doona M, Needham S, Brady RR *et al.*: **Prominent members of the human gut microbiota express endo-acting *O*-glycanases to initiate mucin breakdown**. *Nat Commun* 2020, **11**:4017.

66. • Luis AS, Jin C, Pereira GV, Glowacki RWP, Gugel SR, Singh S, Byrne DP, Pudlo NA, London JA, Basle A *et al.*: **A single sulfatase is required to access colonic mucin by a gut bacterium**. *Nature* 2021, **598**:332-337

The authors characterized the activity of 12 sulfatases involved in the degradation of complex *O*-glycans found in mucins by *Bacteroides thetaiotaomicron*. In particular, one sulfatase (BT1636$^{3S\text{-}Gal}$) was found to play a keystone step in the complex pathway of mucin degradation, as it allowed *B. thetaiotaomicron* to access 3S-Gal-capped *O*-glycans.

67. Larsbrink J, Zhu Y, Kharade SS, Kwiatkowski KJ, Eijsink VG, Koropatkin NM, McBride MJ, Pope PB: **A polysaccharide utilization locus from *Flavobacterium johnsoniae* enables conversion of recalcitrant chitin**. *Biotechnol Biofuels* 2016, **9**:260.

68. Mazurkewich S, Helland R, Mackenzie A, Eijsink VGH, Pope PB, Branden G, Larsbrink J: **Structural insights of the enzymes from the chitin utilization locus of *Flavobacterium johnsoniae***. *Sci Rep* 2020, **10**:13775.

69. Taillefer M, Arntzen MO, Henrissat B, Pope PB, Larsbrink J: **Proteomic dissection of the cellulolytic machineries used by soil-dwelling Bacteroidetes**. *mSystems* 2018, **3**.

70. Kulkarni SS, Zhu Y, Brendel CJ, McBride MJ: **Diverse C-terminal sequences involved in *Flavobacterium johnsoniae* protein secretion**. *J Bacteriol* 2017, **199**.

71. Haitjema CH, Gilmore SP, Henske JK, Solomon KV, de Groot R, Kuo A, Mondo SJ, Salamov AA, LaButti K, Zhao Z *et al.*: **A parts list for fungal cellulosomes revealed by comparative genomics**. *Nat Microbiol* 2017, **2**:17087.

72. Ohara H, Karita S, Kimura T, Sakka K, Ohmiya K: **Characterization of the cellulolytic complex (cellulosome) from *Ruminococcus albus***. *Biosci Biotechnol Biochem* 2000, **64**:254-260.

73. Ding SY, Rincon MT, Lamed R, Martin JC, McCrae SI, Aurilia V, Shoham Y, Bayer EA, Flint HJ: **Cellulosomal scaffoldin-like proteins from *Ruminococcus flavefaciens***. *J Bacteriol* 2001, **183**:1945-1953.

74. • Peng X, Wilken SE, Lankiewicz TS, Gilmore SP, Brown JL, Henske JK, Swift CL, Salamov A, Barry K, Grigoriev IV *et al.*: **Genomic and functional analyses of fungal and bacterial consortia that enable lignocellulose breakdown in goat gut microbiomes**. *Nat Microbiol* 2021, **6**:499-511

The authors of this study employed an approach that combined enrichment cultures and metagenomics to explore how lignocellulosic substrates and antibiotic treatments affect the composition of the rumen microbiome. Reconstruction of the metabolic potential of the microbial communities showed division of labour among herbivore anaerobes; indeed, anaerobic fungi and methanogenic archea enabled production of acetate, formate and methane, whereas bacterially dominated microbiomes mainly produced propionate and butyrate.

75. Yeoman CJ, Fields CJ, Lepercq P, Ruiz P, Forano E, White BA, Mosoni P: **In vivo competitions between *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminoccus albus* in a gnotobiotic sheep model revealed by multi-omic analyses**. *mBio* 2021, **12**.

76. Banerjee S, Schlaeppi K, van der Heijden MGA: **Keystone taxa as drivers of microbiome structure and functioning**. *Nat Rev Microbiol* 2018, **16**:567-576.

77. Ze X, Duncan SH, Louis P, Flint HJ: ***Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon**. *ISME J* 2012, **6**:1535-1543.

78. Belzer C, Chia LW, Aalvink S, Chamlagain B, Piironen V, Knol J, de Vos WM: **Microbial metabolic networks at the mucus layer lead to diet-independent butyrate and vitamin B_{12} production by intestinal symbionts**. *mBio* 2017, **8**.

79. Lindstad LJ, Lo G, Leivers S, Lu Z, Michalak L, Pereira GV, Rohr AK, Martens EC, McKee LS, Louis P *et al.*: **Human gut *Faecalibacterium prausnitzii* deploys a highly efficient conserved system to cross-feed on beta-mannan-derived oligosaccharides**. *mBio* 2021, **12**:e0362820.

80. Naas AEP, Pope PB: **A mechanistic overview of ruminal fibre digestion**. *PeerJ Preprints* 2019, **7**:e27831v1 http://dx.doi.org/10.7287/peerj.preprints.27831v1.

81. Heintz-Buschart A, Wilmes P: **Human gut microbiome: function matters**. *Trends Microbiol* 2018, **26**:563-574.

82. •• Michalak L, Gaby JC, Lagos L, La Rosa SL, Hvidsten TR, Tetard-Jones C, Willats WGT, Terrapon N, Lombard V, Henrissat B *et al.*: **Microbiota-directed fibre activates both targeted and secondary metabolic shifts in the distal gut**. *Nat Commun* 2020, **11**:5773

The authors produced a targeted MDF from wood and showed that its inclusion in pig feed activated a predictable metabolic response in primary (*R. intestinalis*) and secondary (*F. praunsnitzii*) β-mannan degraders as well as other cross-feeders. A multi-omics approach allowed the visualization of the so called 'butterfly effect', whereby the dietary intervention stimulated other non-mannolytic population that were likely taking advantage of underlying availabilities of fermentation intermediates.

83. •• Deehan EC, Yang C, Perez-Munoz ME, Nguyen NK, Cheng CC, Triador L, Zhang Z, Bakal JA, Walter J: **Precision microbiome modulation with discrete dietary fiber structures directs short-chain fatty acid production**. *Cell Host Microbe* 2020, **27**:389-404 e386

The study reports the results of a small randomized controlled trial in humans with three distinctly structured resistant starches. Despite individualized responses, the treatments caused consistent effects on the community structure, with distinct enrichment of certain taxa, and on SCFA production.

84. •• Patnode ML, Beller ZW, Han ND, Cheng J, Peters SL, Terrapon N, Henrissat B, Le Gall S, Saulnier L, Hayashi DK *et al.*: **Interspecies competition impacts targeted manipulation of human gut bacteria by fiber-derived glycans**. *Cell* 2019, **179**:59-73 e13

The authors explore the response of specific human gut taxa in a defined community to specific fibers and use proteomics and forward genetics to identify bioactive nutrients and the mechanisms for their utilization. Notably, the authors fed retrievable bead-based biosensors, composed of fluorescently labeled beads coated with dietary polysaccharides, to germ-free mice colonized with different bacterial combination to evaluate glycan metabolism *in vivo* at a community-wide level.

85. •• Delannoy-Bruno O, Desai C, Raman AS, Chen RY, Hibberd MC, Cheng J, Han N, Castillo JJ, Couture G, Lebrilla CB *et al.*: **Evaluating microbiome-directed fibre snacks in gnotobiotic mice and humans**. *Nature* 2021, **595**:91-95

This study provides a mechanistic understanding of the microbial contribution to human dietary response and show that precise dietary fiber interventions can be used to alter the obese gut microbiomes composition and functions, and ultimately the host's physiological state.

86. Gray CJ, Migas LG, Barran PE, Pagel K, Seeberger PH, Eyers CE, Boons GJ, Pohl NLB, Compagnon I, Widmalm G *et al.*: **Advancing solutions to the carbohydrate sequencing challenge**. *J Am Chem Soc* 2019, **141**:14463-14479.

87. Grabarics M, Lettow M, Kirschbaum C, Greis K, Manz C, Pagel K: **Mass spectrometry-based techniques to elucidate the sugar code**. *Chem Rev* 2021 http://dx.doi.org/10.1021/acs.chemrev.1c00380.